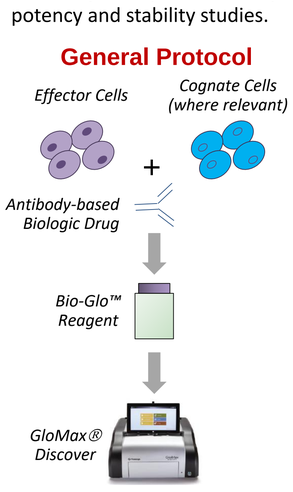
# Releasing the Brakes: Quantitative Cell-based Bioassays to Advance Individual and Combination Immune Checkpoint Immunotherapy

**Jamison Grailer, Julia Gilden, Pete Stecha, Denise Garvin, Jim Hartnett, Frank Fan, Mei Cong and Zhi-jie Jey Cheng**
**Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711**

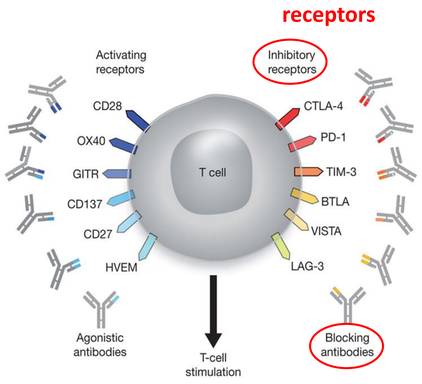
## 1. Introduction

A major challenge in the development of antibody-based biologics drugs is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints that are cumbersome, variable, and often fail to yield data quality required for drug development in a quality-controlled environment. We have developed a portfolio of functional cell-based reporter bioassays to measure the activity of biologics drugs designed to target immune checkpoint receptors including co-inhibitory (e.g. PD-1, CTLA-4, LAG-3) and co-stimulatory (e.g. 4-1BB, GITR, OX40) receptors. These bioassays consist of stable cell lines that express luciferase under the precise control of receptor-mediated intracellular signals. Here we describe the application of MOA-based immune checkpoint co-inhibitory receptor bioassays for biologics drug discovery, development, potency and stability studies.

**General Protocol**

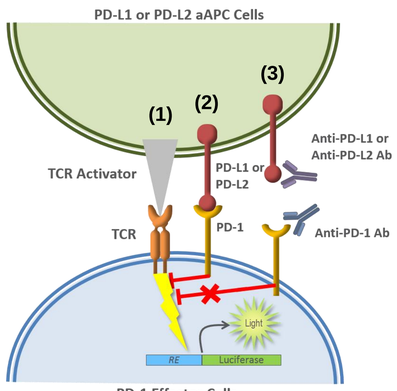



**co-inhibitory receptors**



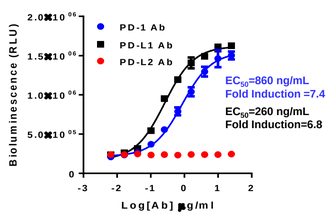

Mellman, et al. (2011) *Nature,* 480:480-89

## 2. PD-1 Blockade Bioassays

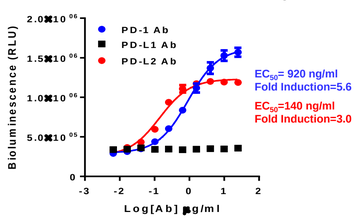



**Assay Design**
(1) TCR engagement induces luciferase activity
(2) Co-engagement of PD-1 with PD-L1 or PD-L2 inhibits luciferase activity
(3) Ab-mediated blockade of PD-1/PD-L1 or PD-1/PD-L2 restores luciferase activity

**PD-1/PD-L1 Blockade Bioassay**




**PD-1/PD-L2 Blockade Bioassay**

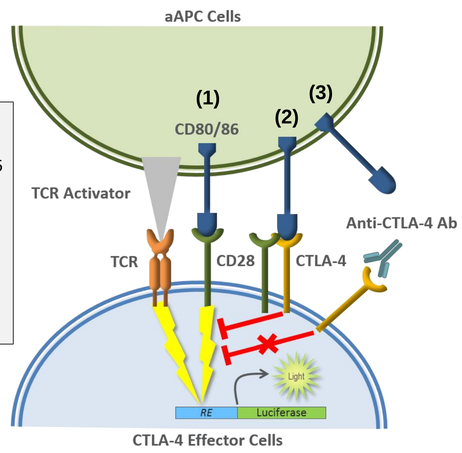



**Left:** TCR-mediated luciferase activity is recovered in the PD-1/PD-L1 bioassay with anti-PD-1 or anti-PD-L1 blocking Abs, but not with anti-PD-L2 blocking Ab.

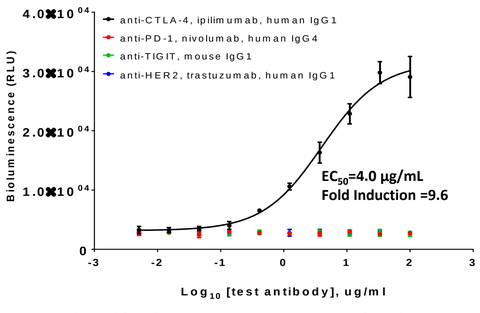
**Right:** TCR-mediated luciferase activity is recovered in the PD-1/PD-L2 bioassay with anti-PD-1 or anti-PD-L2 blocking Abs, but not with anti-PD-L1 blocking Abs.

**All Abs shown here are research grade.**

## 3. CTLA-4 Blockade Bioassay

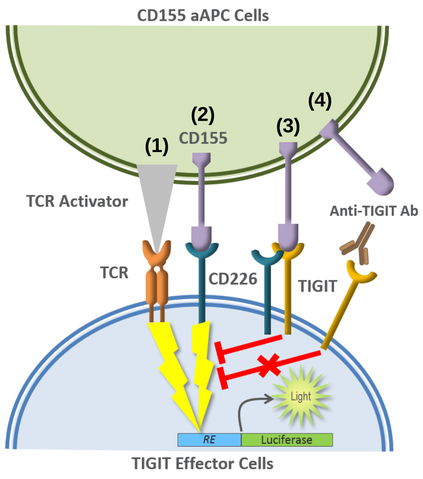



**Assay Design**
(1) TCR activation and CD28 engagement with CD80/86 induce luciferase activity
(2) Engagement of CTLA-4 with CD80/86 inhibits luciferase activity
(3) Ab-mediated blockade of the CTLA-4 interaction restores luciferase activity

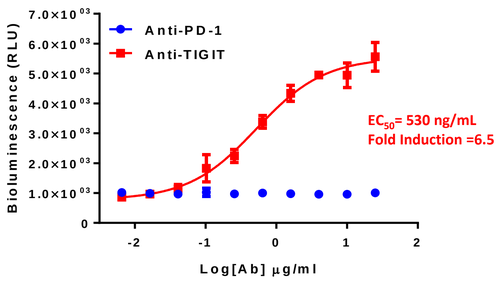



TCR and CD28-mediated luciferase activity is recovered in the CTLA-4 bioassay with an anti-CTLA-4 blocking Ab (ipilimumab), but not with anti-HER2 (trastuzumab), anti-PD-1 (nivolumab) or anti-TIGIT (clone MBSA-43) blocking Abs.

## 4. TIGIT/CD155 Blockade Bioassay

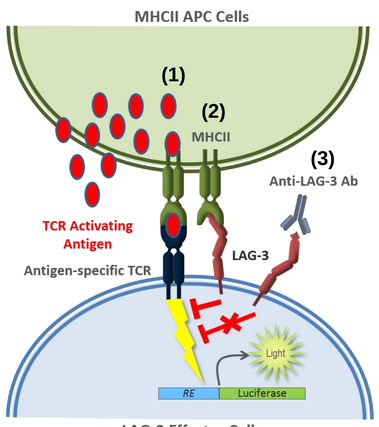



**Assay Design**
(1) TCR engagement induces luciferase activity
(2) When TIGIT is absent, CD226/CD155 provides a co-stimulatory signal
(3) When TIGIT is present, TIGIT competes with CD226 for binding to CD155 and inhibits luciferase activity
(4) Ab-mediated blockade of TIGIT/CD155 restores luciferase activity

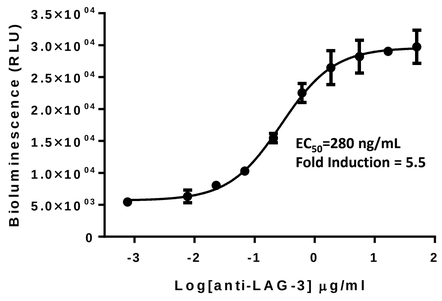



TCR- and CD226-mediated luciferase activity is recovered in the TIGIT/CD155 bioassay with an anti-TIGIT blocking Ab (clone MBSA-43), but not with an anti-PD-1 blocking Ab (nivolumab).

## 5. LAG-3/MHCII Blockade Bioassay

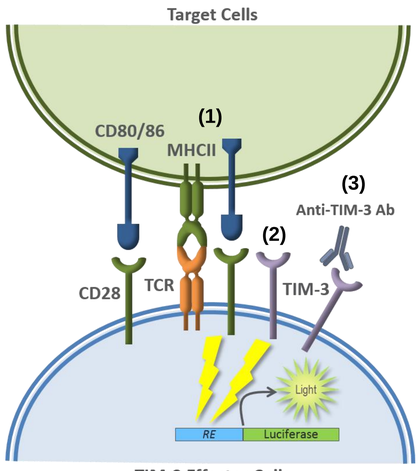



**Assay Design**
(1) Antigen-dependent activation of the TCR induces luciferase activity
(2) Co-engagement of LAG-3 inhibits luciferase activity
(3) Ab-mediated blockade of LAG-3 binding to its ligand restores luciferase activity

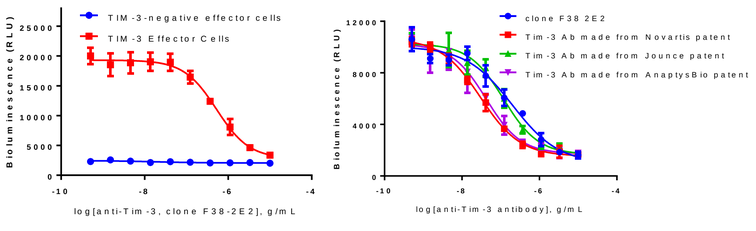



TCR-mediated luciferase activity is recovered in the LAG-3 bioassay with an anti-LAG-3 blocking Ab (clone 17B4).

## 6. TIM-3 Bioassay



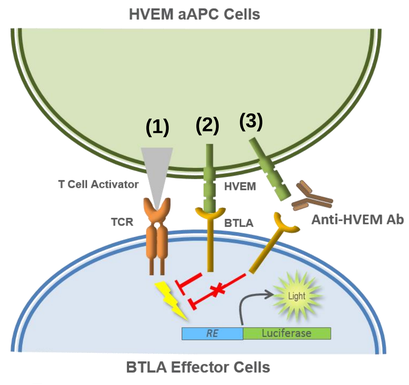

**Assay Design**
(1) TCR activation and CD28 co-stimulation with CD80/86 induce luciferase activity
(2) Expression of TIM-3 enhances TCR/CD28-mediated activation and luciferase activity
(3) Blockade of TIM-3 inhibits luciferase activity



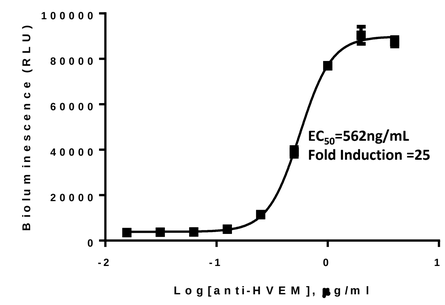

**Left:** TCR/CD28-mediated luciferase activity is enhanced with expression of TIM-3 in effector cells, which is inhibited in a dose-dependent manner with the anti-TIM-3 blocking Ab.

**Right:** TIM-3-mediated luciferase activity is inhibited in a dose-dependent manner with clone F38-2E2 and TIM-3 blocking antibodies made from Novartis, Jounce, and AnaptysBio.

## 7. BTLA/HVEM Blockade Bioassay




**Assay Design**
(1) TCR engagement induces luciferase activity
(2) Co-engagement of BTLA with HVEM inhibits luciferase activity
(3) Ab-mediated blockade of BTLA/HVEM restores luciferase activity

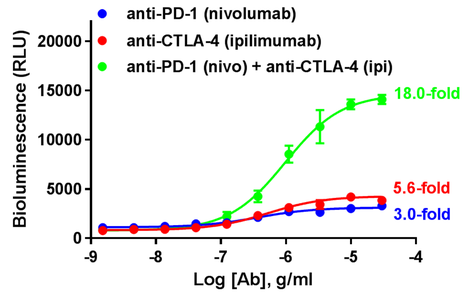



TCR-mediated luciferase activity is recovered in the BTLA/HVEM bioassay with an anti-HVEM blocking Ab (R&D Systems Cat. #AF356, affinity purified goat polyclonal).

***An HVEM/LIGHT co-stimulatory bioassay is also available (data not shown).***

## 8. Combination Bioassays

**PD-1+CTLA-4 Combination Bioassay**

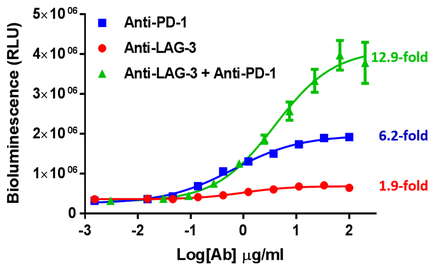



Anti-PD-1 (nivolumab) or anti-CTLA-4 (ipilimumab) blocking Abs induce a 3.0- and 5.6-fold increase in luciferase activity when measured individually. A combination of both Abs induces an 18-fold increase in luciferase activity.

**Assay Design**
(1) PD-L1 and CD80 are expressed on aAPC cells
(2) PD-1, CTLA-4 and CD28 are co-expressed on effector cells
(3) TCR activation and CD80/CD28 engagement induce luciferase activity
(4) Engagement of PD-1/PD-L1 and CD80/CTLA-4 inhibits luciferase activity
(5) Blockade of PD-1/PD-L1 and/or CD80/CTLA-4 restores luciferase activity

**PD-1+LAG-3 Combination Bioassay**

**Assay Design**
(1) PD-L1 and MHC-II are expressed on aAPC cells
(2) PD-1 and LAG-3 are co-expressed on effector cells
(3) TCR activation induces luciferase activity
(4) Engagement of PD-1/PD-L1 and LAG-3/MHC-II inhibits luciferase activity
(5) Blockade of PD-1/PD-L1 and/or LAG-3/MHC-II restores luciferase activity




Anti-PD-1 (clone EH12.2H7) or anti-LAG-3 (clone 17B4) blocking Abs induce a 6.2- and 1.9-fold increase in luciferase activity, respectively. A combination of both Abs induces an 12.9-fold increase in activity.

***Similar results were achieved using the PD-1+TIGIT Combination Bioassay (data not shown).***

## 9. Conclusions

Cell-based reporter bioassays overcome the limitations of primary cell-based assays for functional characterization of antibody and other biologics drugs targeting individual or combination immune checkpoint receptors. Here we show a portfolio of MOA-based bioassays for co-inhibitory immune checkpoint receptors that can be used for antibody screening, characterization, potency and stability studies. These bioassays provide the following:

**Biologically relevant measurement of antibody MOA**

- Specific immune checkpoint regulated expression of luciferase that reflects the native biology of T cell activation.
- Demonstrated ability to measure the potencies of immune checkpoint-targeted antibodies

**Consistent and reliable measure of antibody activity**

- Demonstrated precision, accuracy, reproducibility, robustness
- All assays can be used as “Thaw-and-use” cell format, no cell culture required
- Functional performance suitable for development into potency, stability, and NAb assays

**Easy-to-implement**

- Rapid and convenient workflow
- Amenable to standard 96-well and 384-well plate formats





Corresponding author: Jey.Cheng@promega.com